RESULTS OF INDOOR AIR QUALITY INVESTIGATION

NAVAL SEA SYSTEM COMMAND

WASHINGTON NAVY YARD

BUILDING 201
DECEMBER 2001

CONDUCTED FOR:

NAVSEA

DECEMBER 2001

ADVANCED ENVIRONMENTAL SERVICES, INC.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Naval Sea System Command (NAVSEA) issued a contract to Advanced Environmental Services, Inc. (AESI) for additional indoor air quality testing and to assist in developing criteria for indoor air quality for Building 201at the Navy Yard, Washington. Periodically, occupants complained of sewage or sewer-like odors inside.

During the week of December 3-7, 2001, Dr. David Anderson of AESI toured the facilities and conducted additional sampling. The building had been visited before, and initial samples had been collected.

During this visit, samples were collected for bacteria (possibly from sewage) and Volatile Organic Compounds (VOCs).

The samples were sealed in ice chests and shipped via Fed Ex to the same outside microbiological lab previously used. The preliminary results were received via fax, with the final results received via mail.

On December 4, the outside the air results were found to be 157 Colony Forming Units per Cubic Meter of Air (CFU / M^3) of viable bacteria.

Air samples for bacteria collected from inside Building 201 were lower than the outside air – ranging from 36 to 42 CFU / M^3.

The samples for VOCs were collected from outside and from inside the building for comparison. Several organic compounds were identified at low concentrations. During the visit, it was learned that black plastic vent pipes on the roof were actually soil vents running from the ground through the building and venting to atmosphere; unfortunately, the vents were in fairly close proximity to the air intakes for the building. (This was also the case on Building 201). Two samples were collected from roof vents. More samples would have been collected, had there been more sampling media available. From the roof vents, it was discovered that a halocarbon – Trichlorotrifluoromethane (Freon R-11) was being released into the atmosphere, in addition to Hexane and Methyl Ethyl Ketone and several other organics. This Freon product was also found inside the building.

Verbal reports were issued to Mr. Michael Smith, COTR, with the preliminary data.

The sewage smell is probably due to a sewage treatment plant down the river, and the wind blowing odors into the air intakes on the roof. From the roof of Building 201, the plant could barely be seen beyond the bend in the River.

The report is based on information available to us at this time. No other aspects if indoor air quality (IAQ) were examined. AESI reserves the right to revise, supplement, and otherwise amend our opinions and conclusions, if necessary and warranted by the discovery of new or additional information.

David O. Anderson, Ph.D.
CIH, CSP, QEP, CPEA

January 28, 2002
Date Issued

INTRODUCTION, METHODOLOGIES, AND OBSERVATIONS

Naval Sea System Command (NAVSEA) issued a contract to Advanced Environmental Services, Inc. (AESI) for additional indoor air quality testing and to assist in developing criteria for indoor air quality for Building 201at the Navy Yard, Washington. Periodically, occupants complained of sewage or sewer-like odors inside.

The purpose of the visit was to conduct a visual inspection of the interior, to collect airborne and bulk samples to establish a baseline for Indoor Air Quality measurements, to determine if a possible health risk was present and to recommend appropriate corrective actions.

The investigation was conducted in accordance with the recommendations and guidelines of the American Conference of Governmental Industrial Hygienists (ACGIH), the American Society of Heating, Refrigeration and Air Conditioning Engineers (ASHRAE), the Occupational Safety and Health Administration (OSHA), the National Institute for Occupational Safety and Health (NIOSH), the Environmental Protection Agency (EPA), and established industry standards.

During the week of December 3-7, 2001, Dr. David Anderson of AESI toured the facilities and conducted additional sampling, with the assistance of the COTR, Michael Smith. The building had been visited before, and initial samples had been collected.

During the visit, it was learned that black plastic pipes found on the roof were actually soil vents installed to vent soil gases from under the building. Unfortunately, these vents were within twenty (20) feet or so of the air intakes. It was also learned that the same soil vent arrangement existed for Building 201. The possibility of sanitary sewer gas re-entrainment, vented into the air intake system was examined, but did not appear to be the source of odors.

On December 4, a total of six (6) samples for were collected both inside the building, and outside for comparison. Three (3) air samples were collected for bacteria using Petri dishes (for viable organisms).

The A-6 bioaerosol monitor, used to collect samples onto the Petri dish, was disinfected on-site using isopropyl alcohol. The air-sampling pump had been calibrated prior to the visit for the type of collection media using a standard method – wet test meter.

The samples collected in the Petri, which contained Blood Agar (BAP) media, which allows for both cultivation and differentiation of bacteria, i.e. "viable". Following incubation, the samples are analyzed via light microscopy at 600X magnification, and the data are reported in numbers of Colony Forming Units per Cubic Meter of air (CFU / M^3), as well as the general shapes of the species, such as cocci or bacilli. (Plates were shipped to the lab inside ice chests to minimize growth between collection and laboratory-controlled incubation).

In addition, three (3) samples were collected for total Volatile Organic Chemicals (VOCs) in the air – with two samples collected outside from soil vents, and one inside. These samples used a 400 milliliter evacuated flask equipped with a flow-limiting orifice. Once activated, air was drawn into the prepared flask; following the sampling time, the flask was sealed. Upon arrival at the lab, the flask was purged and contents injected into a gas chromatograph equipped with a mass spectrometer; a total of sixty-three (63) different organic compounds were analyzed for each VOC sample collected.

Following the discovery of the soil gas vent location, attempts were made to obtain additional evacuated flasks for further study; unfortunately, they could not be obtained from the lab in time to complete these studies. Additional testing is suggested in the future.

All samples were sealed and shipped via Fed-Ex to an outside, independent microbiological lab that specialized in identification and analyses of these types of samples; in addition, they also participate in an Environmental Microbiological Proficiency Analytical Testing (EMPAT) quality control program administered by the American Industrial Hygiene Association, designed for maximum quality and control. An affiliate lab that is Accredited by the American Industrial Hygiene Association analyzed the organic materials. Chain-of-Custody forms were maintained. This is the same procedure and labs previously used for NAVSEA investigations.

The preliminary results for the samples were received via fax, followed by mail. (Appendix B). Several telephone conversations were held with NAVSEA discussing the results and procedures.

Results and Discussion

Toxicological and Health Effects

Major Groups of Bacteria

Gram Positive Cocci

Bacteria in this classification are primarily composed of the genera Micrococcus, Staphylococcus, and Streptococcus. Micrococci are common on skin, in soil, dust water and elsewhere and are not considered pathogenic to humans, plants or animals. However, Staphylococcus aureus and many species of Streptococcus are pathogenic and are also found routinely on human skin. Much of the damage caused to tissue infected with these organisms is due to the several different toxins these bacteria produce. The "flesh-eating bacteria" is a strain of Streptococcus that produces a potent toxin responsible for the necrosis of tissue or "flesh eating". Pus formation is also a sign of localized infection with these bacteria. Gram-positive cocci typically represent a large percentage of the bacteria isolated from indoor environments.

Gram Negative

The Gram-negative cocci are primarily of the genera Neisseria and Branhamella, their primary habitat being the mucous membranes of warm-blooded animals. Only two species, Neisseria gonorrhoeae and Neisseria meningitides are considered to be primary pathogens. N. gonorrhoeae is the etiological agent of gonorrhea, the most frequently reported bacterial infection in the United States, occurring in an estimated 3 million individuals each year. N. meningitides causes meningitis and is commonly found in the throat or nasopharynx of asymptomatic individuals who carry the organism, usually only for a period of several weeks. The Gram negative cocci survive very poorly outside of the host, therefore, isolation from the environment is relatively rare.

Bacillus species

Bacillus species bacteria are Gram positive to Gram variable, large, spore forming rods. They are primarily saprophytes that are widely distributed in nature, particularly in soil, dust, and water and on materials of plant and animal origin. The broad range of physiological characteristics within the genus is reflected in a wide range of facultative variants of mesophilic species, facultative and obligate thermophiles, psychrophiles, acidophiles, halophiles etc. that are capable of survival in spore form or even growth in environmental extremes.

The majorities of Bacillus species apparently have little or no pathogenic potential and are rarely associated with disease in humans. The primary exceptions to this are Bacillus anthracis, the etiological agent of anthrax and Bacillus cereus, and a common food borne pathogen. Bacillus subtilis, Bacillus licheniformis and occasionally other Bacillus species have been incriminated in food poisoning. In both cases of anthrax and food poisoning with Bacillus species, symptoms originate from the toxins produced by the bacteria, not from the infection itself.

GRAM POSITIVE NON-SPORULATING BACILLI

The habitats of the Gram positive, non-sporulating bacilli are diverse, ranging from soil and the surface of plants to food and the skin and mucous membranes of mammals. The primary pathogen associated with this varied group is Corynebacterium diphtheriae. This bacteria causes diphtheria, an acute communicable disease manifested by both local infection of the upper respiratory tract and the systemic effect of toxin, which is most notable in the heart and peripheral nerves. Death can result from respiratory obstruction or myocarditis caused by the toxin.

GRAM NEGATIVE BACILLI

The number of species of bacteria in this classification is vast, however, most of these bacteria are associated with moisture sources and tend to be subject to desiccation more than other bacteria. They include organisms such as Escherichia coli, Salmonella, Shigella, Pseudomonas, Klebsiella, Proteus, Enterobacter, Legionella and a number of other genera of bacteria. Their natural habitats range from the intestinal tract of mammals to stagnant and flowing fresh waters, as well as seawater. The types of infection caused by these organisms range from localized infection to fatal septicemia. The disease causing Gram negative bacilli are too numerous for individual discussion in this text. An important aspect of these bacteria that has received recent attention is their ability to produce endotoxin, a component of the cell structure. Endotoxins are the lipopolysaccharide complexes of the cell walls, which are released into the environment when the cell breaks up and are relatively toxic to mammals. Exposure results in fever, malaise, respiratory distress, and so forth. (For detailed explanations, please refer to Appendix C).

AIR SAMPLE RESULTS

Outside:

- Petri Dish: Viable cultures revealed 157 Colony Forming Units per Cubic Meter of Air (CFU / M^3). The breakdown consisted of 40 % Gram Positive Cocci, and 20 % each of Bacillus, Gram Negative and Gram Positive Bacilli.
- VOCs (Roof, Southwest Vent): Of the 63 analyses performed, six (6) compounds were identified above the level of detection for the instruments used. These compounds were Acetone (72 ppb), 2-Proponal (7.5ppb), Toluene (7.2 ppb), Hexane (7.1 ppb), Trichlorofluoromethane (5.2 ppb), and 2-Butanone (Methyl Ethyl Ketone) (2.3 ppb),
- VOCs (Roof, West Vent): Of the 63 analyses performed, four (4) compounds were identified above the level of detection for the instruments used. These compounds were Acetone (57 ppb), 2-Proponal (12 ppb), Toluene (3.4 ppb), and Chloroethane (2.8 ppb).

Third Floor (3W915)

- Petri Dish: Viable cultures revealed 43 Colony Forming Units per Cubic Meter of Air (CFU / M^3) – all Gram Positive Cocci.
- VOCs: Of the 63 analyses performed, seven (7) compounds were identified above the level of detection for the instruments used. These compounds were Acetone (54 ppb), 2-Proponal (6.7 ppb), Trichlorofluoromethane (5.0 ppb), Trichloroethene (4.7 ppb), Toluene (4 ppb), Hexane (3.7 ppb), and 2-Butanone (Methyl Ethyl Ketone) (3.1 ppb).

Third Floor (3E960)

- Petri Dish: Viable cultures revealed 43 Colony Forming Units per Cubic Meter of Air (CFU / M^3) – all Gram Positive Cocci.

First Floor (1E200 - Receiving):

- Petri Dish: Viable cultures revealed 36 Colony Forming Units per Cubic Meter of Air (CFU / M^3) – 36 % Gram Positive Cocci, 29 % Bacillus, and 7 % Gram Positive Bacilli.

CONCLUSIONS

This survey revealed that, on the date of the testing, the overall air quality indoors from a bacteriological standpoint was better than the air quality outside. More VOC compounds were found inside, when compared to outside air, albeit in low concentrations. Acetone is the most common VOC found; however, the presence of Trichlorofluoromethane both outside and inside is of some concern. Trichlorofluoromethane, also known as Freon R-11, is a regulated substance by EPA as an ozone-depleting compound.

RECOMMENDATIONS

Awareness of odors and sources should be made available to all personnel assigned to work in Building 201. The sewage treatment plant downwind is an important contributor to these odors – when the wind is from the right direction.

Periodic monitoring is suggested to examine air quality in relationship to seasonal patterns (temperatures, wind directions, etc), modifications to seating arrangements, the "sealing" of buildings during winter and summer months, the effects of partition heights on airflow, the effects of electronic equipment such as computers on heat loading, and potential water leaks from kitchenettes and filtration units.

A program of training and awareness, including documentation of IAQ "events" should take place, as well.

Even though VOC levels were low, consideration may be given to increasing the ventilation system airflow regarding the number of air exchanges per hour or airflow per person.

Monitoring should be performed on soil vent pipes to determine if these gases are or may be a hazard; the presence of Trichlorofluoromethane – both from the vent stacks and in the building – should be investigated further.

A lead-based paint program including survey, labels, and use of HEPA vacuums should be considered. If lead-based paint is found, a program to seal cracked paint should also be included.

Appendix A

Sampling Locations

Sampling Locations

Sample Number	Sample Type	Location
1	Petri Dish	Outside (Building 197)
2	Petri Dish	3W915
3	VOC	3W915
4	Petri Dish	3E960
5	VOC	Roof, SW vent
6	VOC	Roof, W. Vent
7	Petri Dish	1E200 Receiving

Appendix B

Microbiological Results

And

Lab Data

AEROTECH LABORATORIES, INC.

AESI
1112 Charleston Ct.
Keller, TX 76248
Attn: Dr. D. Anderson

Lab Number: A-112-0840
Project ID: 1245
Date Received: 12/06/01
Date Reported: 12/13/01

AIHA Empat No. 102297

Microbiological Analysis - Air

Lab Number	1			3			6		
Sample Identification	3W915			3E960			1E200 Receiving		
Date Incubated	12/07/01			12/07/01			12/07/01		
Date Analyzed	12/12/01			12/12/01			12/12/01		
Volume (M^3)	0.1400			0.1400			0.1400		
	CFU	CFU/M^3	%	CFU	CFU/M^3	%	CFU	CFU/M^3	%
Viable Bacteria @ 28°C (BAP)	6	43	100	6	43	100	5	36	100
Gram Positive Cocci	6	43	100	6	43	100	4	29	80
Bacillus Species							1	7	20
Gram Positive Bacilli									
Gram Negative Bacilli									
Actinomycete									

Prepared By: CG
CS Review:

Technical Review:
Final Review: Marcela Hodge

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AEROTECH LABORATORIES, INC.

AESI
1112 Charleston Ct.
Keller, TX 76248
Attn: Dr. D Anderson

Lab Number: A-112-0840-02
Project ID: 201/1245
Sample ID: 3W 915 1695
Sample Size: 400 mL Can
Date Received: 12/06/01
Date Analyzed: 12/10/01
Date Reported: 12/19/01

Volatile Organic Compounds (VOC's) - Air

EPA TO14A/TO15

Compound	Results ppbv	Results µg/m³	Comments
1,1,1-Trichloroethane	<2.0	<11.1	
1,1,2,2-Tetrachloroethane	<2.0	<14	
1,1,2-Trichloroethane	<2.0	<11.1	
1,1-Dichloroethane	<2.0	<8.2	
1,1-Dichloroethene	<2.0	<8.1	
1,2,4-Trichlorobenzene	<2.0	<15	
1,2,4-Trimethylbenzene	<2.0	<10	
1,2-Dibromoethane	<2.0	<15.6	
1,2-Dichlorobenzene	<2.0	<12.2	
1,2-Dichloroethane	<2.0	<8.2	
1,2-Dichloropropane	<2.0	<9.4	
1,3,5-Trimethylbenzene	<2.0	<10	
1,3-Butadiene	<2.0	<4.5	
1,3-Dichlorobenzene	<2.0	<12.2	
1,4-Dichlorobenzene	<2.0	<12.2	
1,4-Dioxane	<20	<73.2	
2,2,4-Trimethylpentane	<2.0	<9.5	
2-Butanone (MEK)	3.1	9.3	
2-Hexanone	<2.0	<8.3	
2-Propanol	6.7	16.7	
4-Ethyltoluene	<2.0	<8.8	
4-Methyl-2-pentanone	<4.0	<16.6	
Acetone	54	130.2	
Allyl chloride	<2.0	<6.4	
Benzene	<2.0	<6.5	
Benzyl chloride	<2.0	<10.6	
Bromodichloromethane	<2.0	<13.6	
Bromoethene(Vinyl Bromide)	<2.0	<8.9	
Bromoform	<2.0	<21	
Bromomethane	<2.0	<7.9	
Carbon disulfide	<2.0	<6.3	
Carbon tetrachloride	<2.0	<12.8	
Chlorobenzene	<2.0	<9.4	
Chloroethane	<2.0	<5.4	
Chloroform	<2.0	<9.9	
Chloromethane	<2.0	<4.2	
cis-1,2-Dichloroethene	<2.0	<8	
cis-1,3-Dichloropropene	<2.0	<9.2	
Cyclohexane	<2.0	<7	

Input By: CC
CS Review: SB

Technical Review: SMB
Final Review:

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AEROTECH LABORATORIES, INC.

AESI
1112 Charleston Ct.
Keller, TX 76248
Attn: Dr. D Anderson

Lab Number: A-112-0840-02
Project ID: 201/1245
Sample ID: 3W 915 1695
Sample Size: 400 mL Can
Date Received: 12/06/01
Date Analyzed: 12/10/01
Date Reported: 12/19/01

Volatile Organic Compounds (VOC's) - Air

EPA TO14A/TO15

Results			
Compound	**ppbv**	**µg/m³**	**Comments**
Dibromochloromethane	<2.0	<17.3	
Dichlorodifluoromethane(F-12)	<2.0	<10.1	
Dichlorotetrafluoroethane(F-11)	<2.0	<11.4	
Ethyl Acetate	<2.0	<7.3	
Ethylbenzene	<2.0	<8.8	
Heptane	<2.0	<8.3	
Hexachlorobutadiene	<2.0	<21.7	
Hexane	3.7	13.2	
m&p-Xylene	<4.0	<17.6	
Methyl tert-butyl ether	<4.0	<14.6	
Methylene chloride	<2.0	<7.1	
o-Xylene	<2.0	<8.8	
Propene (Propylene)	<2.0	<3.5	
Styrene	<2.0	<8.6	
Tetrachloroethene	<5.0	<34.5	
Tetrahydrofuran	<4.0	<12	
Toluene	4.0	15.3	
trans-1,2-Dichloroethene	<2.0	<8	
trans-1,3-Dichloropropene	<2.0	<9.2	
Trichloroethene	4.7	25.6	
Trichlorofluoromethane	5.0	28.5	
Trichlorotrifluoroethane	<2.0	<15.5	
Vinyl acetate	<2.0	<7.2	
Vinyl chloride	<2.0	<5.2	

Input By: CC
CS Review: 313

Technical Review: SMB
Final Review: [signature]

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AEROTECH LABORATORIES, INC.

ESI
112 Charleston Ct.
Keller, TX 76248
Attn: Dr. D Anderson

Lab Number: A-112-0840-05
Project ID: 201/1245
Sample ID: Roof W Vent 1461
Sample Size: 400 mL Can
Date Received: 12/06/01
Date Analyzed: 12/10/01
Date Reported: 12/19/01

Volatile Organic Compounds (VOC's) - Air

EPA TO14A/TO15

Compound	Results ppbv	µg/m³	Comments
,1,1-Trichloroethane	<2.0	<11.1	
,1,2,2-Tetrachloroethane	<2.0	<14	
,1,2-Trichloroethane	<2.0	<11.1	
,1-Dichloroethane	<2.0	<8.2	
,1-Dichloroethene	<2.0	<8.1	
,2,4-Trichlorobenzene	<2.0	<15	
,2,4-Trimethylbenzene	<2.0	<10	
,2-Dibromoethane	<2.0	<15.6	
,2-Dichlorobenzene	<2.0	<12.2	
,2-Dichloroethane	<2.0	<8.2	
,2-Dichloropropane	<2.0	<9.4	
,3,5-Trimethylbenzene	<2.0	<10	
,3-Butadiene	<2.0	<4.5	
,3-Dichlorobenzene	<2.0	<12.2	
,4-Dichlorobenzene	<2.0	<12.2	
,4-Dioxane	<20	<73.2	
,2,4-Trimethylpentane	<2.0	<9.5	
-Butanone (MEK)	<2.0	<6	
-Hexanone	<2.0	<8.3	
-Propanol	12	29.9	
-Ethyltoluene	<2.0	<8.8	
-Methyl-2-pentanone	<4.0	<16.6	
Acetone	57	137.4	
Allyl chloride	<2.0	<6.4	
Benzene	<2.0	<6.5	
Benzyl chloride	<2.0	<10.6	
Bromodichloromethane	<2.0	<13.6	
Bromoethene(Vinyl Bromide)	<2.0	<8.9	
Bromoform	<2.0	<21	
Bromomethane	<2.0	<7.9	
Carbon disulfide	<2.0	<6.3	
Carbon tetrachloride	<2.0	<12.8	
Chlorobenzene	<2.0	<9.4	
Chloroethane	2.8	7.5	
Chloroform	<2.0	<9.9	
Chloromethane	<2.0	<4.2	
cis-1,2-Dichloroethene	<2.0	<8	
cis-1,3-Dichloropropene	<2.0	<9.2	
Cyclohexane	<2.0	<7	

Input By: CC
CS Review: JB

Technical Review: SMB
Final Review:

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AEROTECH LABORATORIES, INC.

ESI
112 Charleston Ct.
eller, TX 76248
ttn: Dr. D Anderson

Lab Number: A-112-0840-05
Project ID: 201/1245
Sample ID: Roof W Vent 1461
Sample Size: 400 mL Can
Date Received: 12/06/01
Date Analyzed: 12/10/01
Date Reported: 12/19/01

Volatile Organic Compounds (VOC's) - Air

EPA TO14A/TO15

Results			
Compound	ppbv	µg/m³	Comments
Dibromochloromethane	<2.0	<17.3	
Dichlorodifluoromethane(F-12)	<2.0	<10.1	
Dichlorotetrafluoroethane(F-11)	<2.0	<11.4	
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Ethylbenzene	<2.0	<8.8	
Heptane	<2.0	<8.3	
Hexachlorobutadiene	<2.0	<21.7	
Hexane	<2.0	<7.2	
m&p-Xylene	<4.0	<17.6	
Methyl tert-butyl ether	<4.0	<14.6	
Methylene chloride	<2.0	<7.1	
o-Xylene	<2.0	<8.8	
Propene (Propylene)	<2.0	<3.5	
Styrene	<2.0	<8.6	
Tetrachloroethene	<5.0	<34.5	
Tetrahydrofuran	<4.0	<12	
Toluene	3.4	13	
trans-1,2-Dichloroethene	<2.0	<8	
trans-1,3-Dichloropropene	<2.0	<9.2	
Trichloroethene	<2.0	<10.9	
Trichlorofluoromethane	<2.0	<11.4	
Trichlorotrifluoroethane	<2.0	<15.5	
Vinyl acetate	<2.0	<7.2	
Vinyl chloride	<2.0	<5.2	

Input By: CC
CS Review: SB

Technical Review: SMB
Final Review:

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AEROTECH LABORATORIES, INC.

AESI
1112 Charleston Ct.
Keller, TX 76248
Attn: Dr. D Anderson

Lab Number: A-112-0840-04
Project ID: 201/1245
Sample ID: Roof SW 1441
Sample Size: 400 mL Can
Date Received: 12/06/01
Date Analyzed: 12/10/01
Date Reported: 12/19/01

Volatile Organic Compounds (VOC's) - Air

EPA TO14A/TO15

Compound	Results ppbv	Results µg/m³	Comments
1,1,1-Trichloroethane	<2.0	<11.1	
1,1,2,2-Tetrachloroethane	<2.0	<14	
1,1,2-Trichloroethane	<2.0	<11.1	
1,1-Dichloroethane	<2.0	<8.2	
1,1-Dichloroethene	<2.0	<8.1	
1,2,4-Trichlorobenzene	<2.0	<15	
1,2,4-Trimethylbenzene	<2.0	<10	
1,2-Dibromoethane	<2.0	<15.6	
1,2-Dichlorobenzene	<2.0	<12.2	
1,2-Dichloroethane	<2.0	<8.2	
1,2-Dichloropropane	<2.0	<9.4	
1,3,5-Trimethylbenzene	<2.0	<10	
1,3-Butadiene	<2.0	<4.5	
1,3-Dichlorobenzene	<2.0	<12.2	
1,4-Dichlorobenzene	<2.0	<12.2	
1,4-Dioxane	<20	<73.2	
2,2,4-Trimethylpentane	<2.0	<9.5	
2-Butanone (MEK)	2.3	6.9	
2-Hexanone	<2.0	<8.3	
2-Propanol	7.5	18.7	
4-Ethyltoluene	<2.0	<8.8	
4-Methyl-2-pentanone	<4.0	<16.6	
Acetone	72	173.6	
Allyl chloride	<2.0	<6.4	
Benzene	<2.0	<6.5	
Benzyl chloride	<2.0	<10.6	
Bromodichloromethane	<2.0	<13.6	
Bromoethene(Vinyl Bromide)	<2.0	<8.9	
Bromoform	<2.0	<21	
Bromomethane	<2.0	<7.9	
Carbon disulfide	<2.0	<6.3	
Carbon tetrachloride	<2.0	<12.8	
Chlorobenzene	<2.0	<9.4	
Chloroethane	<2.0	<5.4	
Chloroform	<2.0	<9.9	
Chloromethane	<2.0	<4.2	
cis-1,2-Dichloroethene	<2.0	<8	
cis-1,3-Dichloropropene	<2.0	<9.2	
Cyclohexane	<2.0	<7	

Input By: CC
CS Review: SB

Technical Review: DWB
Final Review:

2020 West Lone Cactus Drive • Phoenix, Arizona 85027 • 623 780-4800 • 1-800-651-4802 • Fax 623 780-7695 • www.aerotechlabs.com

AEROTECH LABORATORIES, INC.

AESI
1112 Charleston Ct.
Keller, TX 76248
Attn: Dr. D Anderson

Lab Number: A-112-0840-04
Project ID: 201/1245
Sample ID: Roof SW 1441
Sample Size: 400 mL Can
Date Received: 12/06/01
Date Analyzed: 12/10/01
Date Reported: 12/19/01

Volatile Organic Compounds (VOC's) - Air

EPA TO14A/TO15

Results			
Compound	**ppbv**	**µg/m³**	**Comments**
Dibromochloromethane	<2.0	<17.3	
Dichlorodifluoromethane(F-12)	<2.0	<10.1	
Dichlorotetrafluoroethane(F-11)	<2.0	<11.4	
Ethyl Acetate	<2.0	<7.3	
Ethylbenzene	<2.0	<8.8	
Heptane	<2.0	<8.3	
Hexachlorobutadiene	<2.0	<21.7	
Hexane	7.1	25.4	
m&p-Xylene	<4.0	<17.6	
Methyl tert-butyl ether	<4.0	<14.6	
Methylene chloride	<2.0	<7.1	
o-Xylene	<2.0	<8.8	
Propene (Propylene)	<2.0	<3.5	
Styrene	<2.0	<8.6	
Tetrachloroethene	<5.0	<34.5	
Tetrahydrofuran	<4.0	<12	
Toluene	7.2	27.6	
trans-1,2-Dichloroethene	<2.0	<8	
trans-1,3-Dichloropropene	<2.0	<9.2	
Trichloroethene	<2.0	<10.9	
Trichlorofluoromethane	5.2	29.6	
Trichlorotrifluoroethane	<2.0	<15.5	
Vinyl acetate	<2.0	<7.2	
Vinyl chloride	<2.0	<5.2	

Input By: CC
CS Review:

Technical Review: SMB
Final Review:

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AEROTECH LABORATORIES, INC.

Thursday, December 13, 2001

Dr. David Anderson
AESI
1112 Charleston Ct.
Keller, TX 76248

Re: Aerotech Project Number A-112-0840

Dear Dr. David:

Aerotech is pleased to provide the enclosed report of analyses for samples submitted Thursday, December 06, 2001. This cover letter and accompanying pages are an integral part of this report. All analyses are performed in our AIHA proficiency-tested laboratory under the FDA Good Laboratory Practice Guidelines and the parameters outlined in the most current version of the American Conference of Governmental Industrial Hygienists Bioaerosol Guidelines. The data generated in this report is based on the samples and accompanying information provided. Aerotech employees did not collect samples for this project, and may provide limited interpretation of this data as it relates to the overall investigation.

Quality Assurance

Aerotech is staffed by certified microbiologists, maintains a rigorous Quality Assurance program and participates in the American Industrial Hygiene Association's Environmental Microbiology Proficiency Testing Program. Our AIHA EMPAT Number is 102297. Aerotech is extremely proud of its excellent scoring in this program and will provide copies of our results upon request. They can also be downloaded from our web site at www.aerotechlabs.com. Below you will find additional information regarding the specific analyses requested for this project.

A003, A004, A005, A006, B002, B003, B004, B007, CC002, CC003, CC004, CC005, S002, S003, S004, S007 W001, W002, W003, W004

Culture Analyses for Fungi and Bacteria

Cultureable microorganisms are those that are viable when media is inoculated, and will grow on the selected media and at the selected temperature. This technique has certain limitations when analyzing for certain types of fungi, specifically *Stachybotrys*. Some reports indicate that the recovery efficiency of *Stachybotrys* spores can be as low as 10% when compared to total spore techniques.

The type of media and incubation temperature can vary depending on the scope of the survey. Isolates are identified to the service level requested. Typical analysis includes identification of most fungi to the genus level. *Aspergillus* and *Penicillium* species are differentiated based on morphology with each variant reported separately. Identification to the species level can be performed if requested in advance. General incubation parameters are summarized below. Incubation times can vary depending on specific growth characteristics. Samples submitted for culture analysis using Cornmeal Agar (CMA) or Cellulose Agar are cultured for 14 days.

Test	Incubation Temperature (° C)	Incubation Time
Environmental Bacteria	28	48 hours
Total Fungi	20-25	7-10 days
Thermophilic fungi	37	7-10 days
Thermophilic Actinomycetes	50	48 hours

Common Culture Media

Acronym	Name
BAP	Tryptic Soy Agar with 5% Sheep Blood
PCA	Plate Count Agar
R2A	R2A
BCYE	Buffered Charcoal Yeast Extract Agar
PDA	Potato Dextrose Agar
MEA	Malt Extract Agar
DG-18	Dichloran Glycerol Agar
SAB	Sabauroud's Dextrose Agar
RBA	Rose Bengal Agar
CYA	Czapeck's Yeast Agar

A010, A010.1, B013

Volatile Organic Compounds (VOC's)

Analysis for VOC's includes the EPA T015 method, utilizing a gas chromatograph (GC) coupled to a mass spectrometer (MS). This method includes quantification of 63 compounds. Tentatively identified compounds (TIC's) can also be identified and their concentrations estimated by performing a compound library search of over 100,000 compounds. Results are reported in parts per billion on a volume basis (ppbv).

For additional information, or if you have any questions regarding this report, please do not hesitate to call.

Sincerely,

Ruth Skinner
Project Manager
Aerotech Laboratories, Inc.
800-651-4802

Analytical References

1. Medically Important Fungi: A Guide to Identification, 3rd ed., ASM, 1995.
2. Standard Methods for the Examination of Water and Wastewater, 19th ed., APHA, 1995.
3. Sampling and Identifying Allergenic Pollens and Molds, Blewstone, 1990.
4. Identifying Filamentous Fungi: A Clinical Laboratory Handbook, Star, 1996.
5. Manual of Clinical Microbiology, 7th ed., ASM, 1999.
6. A Laboratory Guide to Common *Aspergillus* Species and their Teleomorphs, CSIRO, 1994.
7. Bioaerosols: Assessment and Control, ACGIH, 1999.

AEROTECH LABORATORIES, INC.

Precision Analytical Laboratories, Inc.

Date: *18-Dec-01*

CLIENT: Advanced Environmental Services, Inc.
Project: 201/1245
Lab Order: 01120294

CASE NARRATIVE

All analyses included in this report were performed by Precision Analytical Laboratories, Inc. (PAL), 1725 W. 17th Street, Tempe, Arizona (ADHS certification no. AZ0610, California 2410).

PAL participates in the AIHA Proficiency Analytical Testing (PAT) program for metals, solvents, and formaldehyde.

Samples were analyzed using methods outlined in references such as:

Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW846, 3rd Edition.

NIOSH Manual of Analytical Methods, Fourth Edition, 1994. NIOSH Method 7300 analyses are performed using a modified digestion procedure to eliminate the use of perchloric acid. NIOSH Methods 1501 and 1003 are modified to incorporate the use of a mass spectrometer detector instead of FID.

Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air, Second Edition, 1999.

Analytical Comments:

All method blanks and laboratory control spikes met EPA method and/or laboratory quality control objectives for the analyses included in this report.

Sample results have not been corrected for blank values.

If requested, the laboratory can perform a forward library search for non-target peaks and provide additional information about the chemical composition and estimated concentration of the additional peaks. Please contact your project manager for more information.

AEROTECH LABORATORIES, INC.

December 18, 2001

Dr. David Anderson
Advanced Environmental Services, Inc.
1112 Charleston Court
Keller, TX 76248

TEL (817) 379-6968
FAX (817) 337-0615

RE: 201/1245

Order 01120294

Dear Dr. David Anderson:

Precision Analytical Laboratories, Inc. received 3 samples on 12/6/2001 for the analyses presented in the following report.

This report includes the following information:
- Case Narrative.
- Analytical Report: includes test results, report limit (Limit), any applicable data qualifier
  (Qual), units, dilution factor (DF), and date analyzed.
- QC Summary Report.

Ruth Skinner
Project Manager

Appendix C

Bacterial Glossary

BACTERIA

BACTERIA

Bacteria are prokaryotic organisms that do not produce oxygen, even those species that are photosynthetic. Reproduction of bacteria involves growth and binary division, with occasional unequal division and budding. More than 1,700 species of bacteria are known to exist and are classified by morphological, biochemical and more recently, cellular and molecular characteristics.

Nomenclature – The long names often borne by bacteria need not be a source of confusion. They are based on a long-standing binomial system - the first name is that of the genus, the second name that of the species. When written, the genus name is capitalized and the second name, the species name, is not. When printed, both the genus and species name are italicized, e.g. Staphylococcus aureus. A species of microorganisms is a group of which all of the individuals are essentially alike, identifiable and belonging to that specific group. In practice, the identification of bacteria to species is a complex task and usually relatively expensive, especially the identification of environmental organisms with no clinical significance. A genus is a group of similar species. A strain consists of the progeny of a particular group of the same species. For example, Escherichia coli O157 - H7 is particularly pathogenic strain of Escherichia coli. The names of the bacteria are also intended to be descriptive of the features of the organisms. For example - having a general agreement as to what characteristics are possessed by organisms in the genus Bacillus, it saves a great deal of time to use that name in the place of the long list of properties to which it refers, such as "strictly aerobic, spore forming rod-shaped bacterium." Also, by applying the name anthracis to the species of Bacillus causing anthrax, repetitions of long detailed morphological, pathological and clinical descriptions are eliminated. As another example, Salmonella typhi indicates, by general agreement among microbiologists, a facultative, non-spore forming, gram negative, motile, rod-shaped bacterium that does not ferment lactose, does not liquefy gelatin, ferments glucose without gas production and causes typhoid fever.

Classifications

There are four basic morphological types of bacteria - cocci, bacilli, actinomycete and helicoidal forms. Cocci are spherical, bacilli are rod-shaped, actinomycetes are branched and filamentous, and the helicoidal are spiral shaped. These types of bacteria are often further classified by Gram staining in which the different types of bacteria are stained and either classified as Gram positive or negative depending on their ability to hold a specific type of stain. The cocci, bacilli and actinomycetes are commonly found in the indoor environment and the helicoidal forms are rarely found.

Bacteria are also classified as either aerobic or anaerobic. Aerobic bacteria require oxygen to survive and reproduce. Anaerobes require oxygen deficient and often carbon dioxide enriched atmospheres to proliferate. Strict anaerobes are of little concern in indoor air quality investigations with the exception of facilities with areas of decreased oxygen atmospheres such as in wastewater treatment plants and composting facilities.

For the purposes of most indoor air quality investigations, classification of aerobic bacteria by Gram stain morphology is sufficient. Special circumstances may require

more extensive testing and analysis. These circumstances should be discussed with the laboratory prior to sampling so that proper sampling techniques and analyses are conducted to meet those requirements.

Major Groups of Bacteria

Gram Positive Cocci

Bacteria in this classification are primarily composed of the genera Micrococcus, Staphylococcus, and Streptococcus. Micrococci are common on skin, in soil, dust water and elsewhere and are not considered pathogenic to humans, plants or animals. However, Staphylococcus aureus and many species of Streptococcus are pathogenic and are also found routinely on human skin. Much of the damage caused to tissue infected with these organisms is due to the several different toxins these bacteria produce. The "flesh-eating bacteria" is a strain of Streptococcus that produces a potent toxin responsible for the necrosis of tissue or "flesh eating". Pus formation is also a sign of localized infection with these bacteria. Gram-positive cocci typically represent a large percentage of the bacteria isolated from indoor environments. Their thick walled spherical morphology and carotenoid pigments affords them protection from ultraviolet light and desiccation.

Staphylococcus

Most Staphylococcal species are common inhabitants of the human skin and mucous membranes. Some exhibit niche preferences. For example Staphylococcus capitis is found in large populations on the human head, especially the scalp and forehead where sebaceous glands are numerous and well developed. Staphylococcus aureus prefers the anterior nostrils as a habitat. Skin infections caused by Staphylococcus aureus are the most common human staphylococcal infections. These include cellulitis, pustules, boils, carbuncles, impetigo and postoperative wound infections. A common community acquired disorder is food poisoning caused by thermo stable enterotoxins produced in foods during growth of S. aureus. In the late 1950s and early 1960s S. aureus caused considerable mortality and morbidity as a nosocomial (hospital acquired) pathogen. The advent and use of semi-synthetic penicillins in the intervening years have provided successful therapy for serious S. aureus infections, however, methicillin resistant S. aureus strains have recently emerged as major clinical and epidemiological concern in hospitals. A community-acquired disease of potentially serious consequences, toxic shock syndrome, also has been attributed to infection or colonization with S. aureus. Other common staphylococcal pathogens are S. epidermidis, S. saprophyticus, S. Intermedius and S. hyicus.

Streptococcus

Humans are the natural reservoir for the beta-hemolytic group A streptococcus, Streptococcus pyogens, and the etiological agent of "Strep throat". Transmission from person to person is frequently associated with close contact with an asymptomatic carrier. Contaminated food may also serve as a vehicle for infecting a large number of people. S. pyogens is a known cause of tonsillitis, pharyngitis, sinusitis, lymphadenitis, pyoderma, bacteremia, arthritis, osteomyelitis, endocarditis and meningitis. Children are usually more severely affected than healthy adults are. They usually become ill with fever, sore throat and tonsillitis. The carrier state develops in 25% of patients despite antibiotic therapy. Some strains produce scarlet fever in which the infection is accompanied by a rash. In severe cases rheumatic fever may occur. Streptococcus

pneumoniae is the most frequent cause of otitis media and bacteremia in infants and children. It is the major cause of community acquired bacterial pneumonia with approximately a half a million cases reported each year in the United States alone. The organism is frequently isolated from the respiratory tract of healthy individuals. Infants have the highest rate of pneumococcal infection, however, individuals at risk include the immune suppressed and individuals with chronic lung conditions.

GRAM NEGATIVE

Cocci The Gram-negative cocci are primarily of the genera Neisseria and Branhamella, their primary habitat being the mucous membranes of warm-blooded animals. Only two species, Neisseria gonorrhoeae and Neisseria meningitides are considered to be primary pathogens. N. gonorrhoeae is the etiological agent of gonorrhea, the most frequently reported bacterial infection in the United States, occurring in an estimated 3 million individuals each year. N. meningitides causes meningitis and is commonly found in the throat or nasopharynx of asymptomatic individuals who carry the organism, usually only for a period of several weeks. The Gram negative cocci survive very poorly outside of the host, therefore, isolation from the environment is relatively rare.

BACILLUS SPECIES

Bacillus species bacteria are Gram positive to Gram variable, large, spore forming rods. They are primarily saprophytes that are widely distributed in nature, particularly in soil, dust, and water and on materials of plant and animal origin. The broad range of physiological characteristics within the genus is reflected in a wide range of facultative variants of mesophilic species, facultative and obligate thermophiles, psychrophiles, acidophiles, halophiles etc. that are capable of survival in spore form or even growth in environmental extremes.

The majorities of Bacillus species apparently have little or no pathogenic potential and are rarely associated with disease in humans. The primary exceptions to this are Bacillus anthracis, the etiological agent of anthrax and Bacillus cereus, and a common food borne pathogen. Bacillus subtilis, Bacillus licheniformis and occasionally other Bacillus species have been incriminated in food poisoning. In both cases of anthrax and food poisoning with Bacillus species, symptoms originate from the toxins produced by the bacteria, not from the infection itself.

GRAM POSITIVE NON-SPORULATING BACILLI

The habitats of the Gram positive, non-sporulating bacilli are diverse, ranging from soil and the surface of plants to food and the skin and mucous membranes of mammals. The primary pathogen associated with this varied group is Corynebacterium diphtheriae. This bacteria causes diphtheria, an acute communicable disease manifested by both local infection of the upper respiratory tract and the systemic effect of toxin, which is most notable in the heart and peripheral nerves. Death can result from respiratory obstruction or myocarditis caused by the toxin.

GRAM NEGATIVE BACILLI

The number of species of bacteria in this classification is vast, however, most of these bacteria are associated with moisture sources and tend to be subject to desiccation more than other bacteria. They include organisms such as Escherichia coli, Salmonella, Shigella, Pseudomonas, Klebsiella, Proteus, Enterobacter, Legionella and a number of other genera of bacteria. Their natural habitats range from the intestinal tract of mammals to stagnant and flowing fresh waters, as well as seawater. The types of

infection caused by these organisms range from localized infection to fatal septicemia. The disease causing Gram negative bacilli are too numerous for individual discussion in this text. An important aspect of these bacteria that has received recent attention is their ability to produce endotoxin, a component of the cell structure. Endotoxins are the lipopolysaccharide complexes of the cell walls, which are released into the environment when the cell breaks up and are relatively toxic to mammals. Exposure results in fever, malaise, respiratory distress, headaches, alterations in white blood cell counts and death.

ACTINOMYCETES

The actinomycetes are a distinct group of microorganisms defined by morphological criteria, basically their ability to grow as branching, filamentous cells that either form spores or reproduce by fragmentation of hyphae. Because of their resemblance to fungi, the actinomycetes were once considered members of the Fungi Imperfecti. It has been proven, however, that these organisms are not eukaryotic fungi, but prokaryotic bacteria. Most aerobic actinomycetes represent part of the indigenous microflora found in soil, mud and dust; on the surfaces of vegetation; within decaying vegetation; in composts; in both fresh and marine water; and in decaying animal feces. All of the medically important actinomycetes have been isolated from environmental reservoirs worldwide. Most actinomycetes are mesophilic, however, thermophilic groups abound in manure and compost piles.

MESOPHILIC ACTINOMYCETES

The most clinically significant actinomycetes belong to the genus Nocardia, of which there are currently 12 accepted species. All of the infections in humans can be divided into the following six categories - 1. Pulmonary nocardiosis; 2. Systemic nocardiosis; 3. Central nervous system nocardiosis; 4. Cutaneous, subcutaneous, and lymphocutaneous nocardiosis; 5. Extrapulmonary localized nocardiosis; 6. Nocardial mycetoma. In the United States the most common from of disease is pulmonary nocardiosis caused by Nocardia astereroides. In tropical regions, mycetoma caused by N. brasiliensis is most frequently diagnosed. A mycetoma is a chronic granulomatous disease that begins as a painless nodule at the site of a localized injury, such as a puncture wound from a thorn. With time the nodule increases in size and becomes purulent and necrotic, producing drainage tracts that expand into surrounding tissue, ultimately invading muscle and adjacent bones causing osteomyelitis.

The incidence of nocardial infections in humans is not known, as few attempts to determine the prevalence of nocardiosis have been made. Several reports indicate that infections by these bacteria are not rare, are frequently misdiagnosed or are under diagnosed, and that the incidence of infection is increasing. The spectrum of disease caused by nocardia is broad and varies from a self limited, asymptomatic infection to an aggressive, destructive disease resulting in death. Every category of nocardial infection described above has been diagnosed in previously healthy adults. However, the nocardiae are frequently being recognized as emerging opportunistic pathogens; the most common underlying predispositions include organ transplantation, malignancies, use of corticosteriods, alcohol abuse, diabetes and other debilitating factors.

THERMOPHILIC ACTINOMYCETES

Allergic respiratory disease caused by the actinomycetes is referred to as farmer's lung, a hypersensitivity reaction to repeated exposure to antigens produced by the actinomycetes, particularly the thermophiles. The most common actinomycetous agents

of farmer's lung are Micropolyspora faeni, Thermoactinomyces vulgaris and Thermoactinomyces vulgaris. These organisms do not represent all of the possible etiological agents of the syndrome, however, they represent a majority of the cases reported. Thermophilic actinomycetes are usually found in closed barns, silos, grain mills, bagasse (sugar cane waste) and poorly maintained air conditioning ducts.

LEGIONELLA

The medical significance of the genus Legionella was first recognized following an outbreak of pneumonia among members of an American Legion convention in 2016. Since that time there have been many recorded outbreaks, as well as individual cases, of legionellosis. In many respects Legionella bacteria and Legionnaires' Disease are progeny of the technology age. Technology has facilitated many of the epidemics by providing means for efficient transmission of bacteria and an increasing number of susceptible hosts due to advancements in medicine. The genus Legionella contains over two-dozen species of which about half have been implicated in human disease. Of all the pathogenic species, Legionella pneumophila is by far the most commonly isolated from afflicted individuals. There are two distinct forms of legionellosis. The first and foremost is Legionnaires' disease, a progressive and potentially fatal pneumonia caused by the inhalation of water aerosols containing Legionella bacteria deep into the lung. The onset is relatively abrupt, with high fever, malaise, myalgia, headache and nonproductive cough. Legionnaires' Disease has a 20% fatality rate. Standard treatment is antibiotic therapy with erythromycin. The second form of legionellosis, called Pontiac Fever is a self-limiting non-pneumonic illness characterized by fever and flu-like symptoms. It is believed that Pontiac Fever symptoms are not a result of actual infection, but are a toxic reaction to exposure to very high levels of the bacteria. The attack rate for Legionnaires' Disease is less than 5% while the attack rate for Pontiac Fever is greater than 95%.

Legionella is not contagious. Transmission to humans occurs through the inhalation of Legionella contaminated water aerosols. There is considerable controversy concerning the public health benefits of monitoring certain environments for Legionella bacteria, as Legionella are often present in water systems in the absence of disease. There is general agreement that monitoring is warranted in order to identify the source of an outbreak, to evaluate the efficacy of biocides or to monitor areas where particularly susceptible people may frequent. Even if monitoring is not performed, aggressive maintenance and disinfection procedures should be implemented for devices known to transmit Legionella. Examples of the types of institutions that have implemented Legionella prevention and monitoring programs include hospitals and health care clinics, industrial facilities, hotels, retirement communities, public facilities and schools. The Legionella are thin Gram-negative bacilli measuring 0.3 to 0.9 microns by 1.5 to 5 microns. Legionella species are nutritionally fastidious, requiring the amino acid L-cysteine for growth on laboratory media. Their natural habitat includes waters worldwide, specifically surface and potable waters and very moist environments. It is well known that other environmental microorganisms are important for the survival and growth of Legionella in the environment, because inclusion of the complex microbiota found in tap water is necessary to cultivate Legionella pneumophila in water suspensions in vitro. An increasing body of evidence has supported the importance of protozoa in the proliferation of Legionella bacteria in the environment. The bacteria multiply within the protozoa, much as they do within human macrophages, enabling them to pass through domestic water treatment systems to colonize individual distribution systems downstream. Heavy colonization can occur in low flow plumbing

fixtures, stagnant water lines, dead end legs, plumbing fixtures, hot water systems, whirlpool spas and cooling towers.

MYCOBACTERIA

The genus Mycobacterium requires special consideration in the sampling of indoor environments. Typically sampling for mycobacteria in indoor settings occurs only under special circumstances, specifically when a route for transmission of mycobacteria is suspected. Species within the genus include the primary pathogens Mycobacterium tuberculosis, the etiological agent of tuberculosis, Mycobacterium bovis (also causes tuberculosis), Mycobacterium leprae, Hansen's disease or leprosy and Mycobacterium avium in addition to over a dozen other species associated with disease in humans. The mycobacteria are slightly curved or straight non-motile aerobic bacilli. Their growth rates for mycobacteria are slow to very slow with generation time ranging from 2 hours to greater than 20 hours. Optimal temperatures for growth vary between the species, ranging from 30¡C to almost 45¡C. Most species can be readily cultured using specialized media, however the incubation time can exceed 6 weeks. The exception to this is M. leprae, which cannot be cultured outside of living cells.

Most species of mycobacteria are free living in soil and water, but the major ecological niche of the primary pathogens is diseased tissue of warm-blooded animals, including humans.

MYCOBACTERIUM TUBERCULOSIS

Tuberculosis is a major global health problem. There are an estimated 8 million new cases and 3 million deaths attributed to the disease annually. This makes tuberculosis the leading cause of death in the world due to a single infectious agent. In the United States the incidence of tuberculosis declined steadily until 1985, when the downward trend reversed. A number of factors have influenced the recent rise of tuberculosis cases in the United States including multi-drug resistant strains of the bacterium, the advent of the AIDS epidemic, immigration from endemic areas and transmission in high-risk environments. M. tuberculosis bacteria are more common in today's urban environment than most people believe. It is so widespread, especially in city streets, public transportation, theaters and any place where people congregate, that no one in an urban community escapes contact with the organism. It is well known that up to 80 percent of individuals living in urban populations have been infected with M. tuberculosis at some time in their lives. Healed pulmonary tuberculosis lesions are found in the lungs of a large percentage of adults upon whom autopsies have been performed following death due to unrelated causes. Tuberculosis is primarily an airborne disease. M. tuberculosis is carried in airborne particles known as droplet nuclei that are generated when patients with pulmonary tuberculosis cough. It is estimated that a single patient who is raising a considerable amount of sputum may discharge up to three billion bacilli in a 24-hour period. Contact with infected individuals is a particular concern for infants as infection may proceed to generalized, acute, fatal infection with meningitis. Adults and children over five are more resistant. Following inhalation of the bacteria a tissue reaction occurs in the lung that results in the formation of a lesion called a tubercle, one or more bacilli surrounded by a small mass of pus and phagocytes. Later they become enclosed within distinctive multinucleate tissue cells surrounded by connective tissue cells. Typically this lesion will heal without further progress and without remarkable symptoms of any kind, although the bacilli may remain viable for many years.

When bacterial virulence is very high, or dosage large and continuous, or immune resistance low, the bacilli continue to multiply, enlarging the tubercles and killing the tissue at the center, which becomes coagulated in a cheesy mass. This is called caseation. If the process continues unabated, individual caseated masses expand into one another to form on large, caseous mass. There is a delicate balance between health and disease in tuberculosis. Generally anything that diminishes the immune systems ability to combat disease, increases the individual's susceptibility to tuberculosis, whether it be to a new exposure to fresh bacilli or reactivated tubercle involvement in the lung. The danger of active infection always lurks for the malnourished, alcoholics, drug addicts and anyone with a debilitating disease.

MYCOBACTERIUM LEPRAE

The principal manifestations of leprosy include numbing of the extremities and peripheral nerves accompanied by skin lesions. There are an estimated 10 to 12 million active leprosy cases worldwide, primarily in Asia and Africa. Small pockets of infection exist in the United States in Texas and Louisiana, with the primary non-human reservoir suspected to be the nine-banded armadillo. M. leprae cannot be cultured in vitro.

MYCOBACTERIUM AVIUM

Mycobacterium avium is ubiquitous in nature and has been isolated from water, soil, plants, house dust and a myriad of other environmental sources. It is also shed in large numbers in the feces of infected exotic birds and domestic poultry. The organism is of low pathogenicity in healthy adults, frequently colonizing individuals without causing disease. The occurrence of infections due to M. avium has increased significantly since the advent of AIDS. Prior to the AIDS epidemic most cases involved pulmonary infection, similar to tuberculosis, in individuals with preexisting lung disease such as chronic bronchitis, emphysema, healed tuberculosis, etc. Disseminated Mycobacterium avium infections occur in about 10% of AIDS patients and severely affect the quality of life and long-term survival of these patients.